from the study in humans by SONG *et al*. [1] and our *in vivo* studies may provide an important finding regarding difference in susceptibility to nanoparticles between humans and rodents. The second point to be addressed is the possibility of endotoxin attaching to (around) the nanoparticles. Do the authors have any information about endotoxin level in the lung samples or endotoxin concentration in the workplace? We have previously demonstrated that combined exposure to nanoparticles and endotoxin elicited devastating lung injury compared to nanoparticles or endotoxin alone [4]. The lethal events occurring in the patients may have resulted from synergistic effects of nanoparticles with other toxic substances, rather than effects of the nanoparticles alone. Finally, we would like to know if these seven patients had pre-existing atopy. In particular, we are interested in the physical condition of the patients described in ''The general characteristics of the patients'' section in which ''all patients suffered from rash with intense itching on their faces'' [1], implicating an association with some immunological impairment. Allergen-specific immunoglobulin (Ig)E titres examined by radioallergosorbent test could provide hints for detection, as total IgE (measured by radioimmunosorbent test) does not always cover allergic conditions. Furthermore, atopic subjects are prone to particulate matter (PM), *i.e.* PM exposure significantly exacerbates the pathophysiology in the subjects [2, 5], sometimes leading to a fatal outcome. A careful search for pre-existing illness (in particular, atopy) may have helped in resolving the pathogenesis of the lung damage seen in the patients. In any case, the study by SONG *et al*. [1] is valuable for future inhalation toxicology, and environmental and preventive medicine, if correlated to previous *in vivo* findings.

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From the authors:

We would like to thank K. Inoue and H. Takano for their comments and questions regarding our paper entitled ''Exposure to nanoparticles is related to pleural effusion, pulmonary fibrosis and granuloma'' [1].

We read with great interest the study mentioned by K. Inoue and H. Takano [2] with the knowledge that combined exposure to nanoparticles and endotoxin elicited devastating lung injury in comparison to nanoparticles or endotoxin alone. However, with regard to the patients in our study, no evidence was found to show that their symptoms might be related to endotoxin from their occupational exposure, our on-the-spot investigation, clinical observations and examinations, or long-term follow-up. If the patients had inhaled the endotoxin, some symptoms and signs of ''endotoxin poisoning'' would exist, such as fever, tiredness, headache or hypotension, but none of these were observed.

In general, two mechanisms are involved in the toxicity of nanoparticles. One mechanism is that nanoparticles themselves directly exert toxicities, which are related to the chemical component, size and shape of nanoparticles [3]. When some nanomaterials gain entry into the body, either *via* inhalation, dermal or oral routes, and penetrate into cells, they can subsequently pose a series of cytotoxicities or promote DNA damage by several mechanisms [4]. For example, nanoparticles can physically interact with the DNA molecule or proteins, which may lead to physical damage to the cell or genetic material. In addition, inflammation and oxidative stress (generation of reactive oxygen species) induced by nanoparticles have been identified as giving rise to effects on cell membranes, cytoplasm, nuclei and mitochondrial function [4, 5]. Importantly, nanoparticles can damage cells through the regulation of redox-sensitive transcription factors, induction of apoptotic and necrotic cell death and decreased proliferation, and DNA damage responsive signalling [4–6]. A recent study has shown that cationic starburst polyamidoamine dendrimer (PAMAM) nanoparticles trigger autophagic cell death by deregulating the Akt-TSC2-mTOR signalling pathway, and induce acute lung injury *in vivo* [7]. The other mechanism of nanoparticle toxicity is that nanoparticles can be used as delivery carriers [8]. In cancer and gene therapy, nanoparticles can deliver drugs at high concentrations to the sites of interest, *e.g.* cancer lesions. It is not difficult to understand that if the material the nanoparticles carry is not a drug but highly toxicant, it may cause potential damage to cells.

In our study, ''nanoparticles were observed to lodge in the cytoplasm and caryoplasm of pulmonary epithelial and mesothelial cells'' [1], direct interaction between nanoparticles and the DNA molecule or DNA-related proteins may lead to physical damage to the genetic material. In addition, nanoparticles may exert toxicities on cells by other mechanisms, such as inflammation, oxidative stress or cell responsive signalling. In order to question whether the lethal events resulted from the synergy of nanoparticls with other toxic substances, or to what extent the illnesses were due to particles and other toxic substances, further animal experiments need to be performed in order to draw a firm conclusion. However, in our opinion, as the workers were used the exposure of powder coatings, as well as other reasons

discussed in the study [1], the major reason for illness may be due to the powder in the coating, which contains nanoparticles.

As for the rashes the patients experienced on their faces, these may show the physical damage caused by polyacrylate nanoparticles to skin. It is not appropriate to link them to pre-existing atopy or immunological impairment. The rashes on the face of patients, characterised by skin itching, pachylosis and breakage, varied greatly from the rashes experienced in patients with immune-related disorders, such as systemic lupus erythematosus, which were characterised by no inching but smooth and intact skin. Moreover, detailed clinical examinations and follow-up excluded the immune-related disorders or pre-existing atopy.

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To the Editors:

We are writing to express concern about the recent paper by SONG *et al.* [1], which was published in a recent issue of the *European Respiratory Journal*. We read the paper with great interest, as it would constitute the first report of human nanoparticle-related disease and death. The title attracted our attention, as well as that of the international media: ''Exposure to nanoparticles is related to pleural effusion, pulmonary fibrosis and granuloma.'' Note the use of the word ''is.'' The title appears to state a fact. We object.

We write as individuals committed to the science of nanotoxicology. It is known that nanomaterials have unusual physical and chemical properties [2] and that these unusual characteristics, combined with the ever expanding use of nanomaterials, deserve careful investigation [3, 4]. It is not the time for complacency. Serious methodical investigations of potential toxicity from nanoparticles and nanofibers are clearly warranted. We believe wholeheartedly in the cautionary principle and in providing the best possible data to protect workers and consumers.

This paper, however, draws premature conclusions and does not add objective evidence permitting us to evaluate the possibility of adverse health effects of nanoparticles in humans [1]. We do not agree with the implied conclusion that it was nanoparticles which caused the pulmonary problems and the deaths of two workers. The study by SONG *et al.* [1] clearly describes a primitive workplace characterised by a total lack of even the most rudimentary precautions. In the face of dangerous activities, including air spraying and curing polymers with heat, exposure controls were absent. SONG *et al.* [1] state, ''It is estimated that the air flow or turn over rates of indoor air would be very slow, or quiescent due to the lack of windows and the closed door''.

Moreover, adequate exposure assessment is utterly lacking. We are especially concerned about a variety of hazards in this workplace. Yes, there is some evidence for the existence of nanoparticles, but the list of chemicals and dusts to which these workers were exposed is extensive. Many chemicals were used in this space where ventilation was inadequate or even absent. During spraying and related procedures, indoor concentrations of a variety of toxic materials were probably very high. All seven females who worked there for 5–13 months had a variety of symptoms; there is convincing evidence of that. The title of this article should have read, ''Poor working conditions cause pleural effusion, pulmonary fibrosis and granuloma''. It would have been appropriate if the presence of nanoparticles had been mentioned, and even speculations made that they may have contributed to the resulting pathology. But the current message that nanoparticle exposure is primarily responsible is not warranted. SONG *et al.* [1] fail to provide any information about other respirable toxic agents which were inhaled both as solid aerosols and in the gas phase. Hence, the conclusion that nanoparticles are causally related to these pulmonary diseases is much too premature and scientifically not acceptable.

The outcome is certainly tragic and deserves the attention of the public and the international community of chest physicians, pneumologists and lung biologists. But this study primarily emphasises the importance of implementing appropriate industrial hygiene practices. It fails to provide evidence about unusual risks posed by nanoparticles. We simply do not know to what extent exposures to nanoparticles in this workplace contributed to the evident pulmonary pathology and to the documented unfortunate consequences.

We encourage careful studies of nanotoxicology, both in the laboratory and in the workplace. But we ask that excellent